REGULATION OF CELLULASE AND CELLOBIASE IN *Neurospora crassa*

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In *Neurospora crassa* the *cell-1* mutant exhibits a constitutive production of the two enzymes, cellobiase and cellulase. Aryl-beta-glucosidase levels are not significantly altered by the presence of the *cell-1* gene. This observation is consistent with the assignment of the sole regulation of aryl-beta-glucoside to the *gluc-1* gene.

The degradation of cellulose to glucose in *Neurospora crassa* appears to be a sequential process involving the participation of two distinct enzymes: a cellulase and a cellobiase (Eberhart, *et.al.*, 1964). Both enzymes attack beta-glucosidic linkages but each can be distinguished by a characteristic activity with carboxymethyl cellulose (CMC). Cellulase lowers the viscosity of solutions of CMC by acting as an endocellulase (Reese, *et.al.*, 1950). Cellobiase does not have this ability but does exhibit exocellulase activity and a high activity toward cellobiose. A third enzyme, aryl-beta-glucosidase, has also been isolated from Neurospora mycelia and conidia (Mahadevan and Eberhart, 1964). The simultaneous induction of all three enzymes by cellobiose (Eberhart, *et.al.*, 1964), suggested a coordinated regulatory mechanism analogous to that described for beta-galactosidase

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system of E. coli (Jacob and Monod, 1961). This report is concerned with the simultaneous production of cellobiase and cellulase as a regulatory expression of the cell-1 mutation in Neurospora; aryl-beta-glucosidase levels are not affected by the cell-1 locus.

Materials and Methods: For quantitative studies of enzymatic activities, mycelia were collected, washed, extracted by grinding (Horowitz and Shen, 1952) in chilled 0.01M phosphate buffer (pH 7.1), and centrifuged for 40 minutes at 10,000 rpm at 4°C to remove cell debris. The supernatent solution was frozen prior to assay. A discontinuous method for beta-glucosidase assay determined the total beta-glucosidase activity aryl-beta-glucosidase (thermostable) and the cellobiase (thermolabile) components were distinguished by heating the extracts for one minute at 60°C prior to assay (Eberhart, et.al., 1964). The cellulase activity of mutant extracts was determined by the decline of the viscosity of a solution of carboxymethyl cellulose (4M, Hercules Powder Co.) in an Ostwald viscosimeter. The specific activity of cellulase was defined by $10 \times \eta_{rel}$/mg protein. Protein determination was by the biuret method (Gornall, et.al., 1949). The specific activity of the beta-glucosidase was defined as change in O.D. at 410 mμ x 10^4 per 10 min. per mg protein.

Mutant selection: The initial cell-1 mutant was isolated from a strain that was genotypically cell-1^+, gluc-1, and therefore low in aryl-beta-glucosidase activity (Mahadevan and Eberhart, 1964). After irradiation of the cell-1^+, gluc-1 strain several thousand strains were isolated from single conidia and tested for their ability to destroy the beta-glucoside esculin (Eberhart, et.al., 1964). The presence of the gluc-1 gene in these isolates facilitated detection of changes due to cellobiase activity. Since both aryl-beta-glucosidase and cellobiase will attack esculin, lowered aryl-beta-glucosidase (gluc-1) reduced the interference due to this enzyme's over lapping specificity. The new gluc-1, cell-1 strain was selected for its unusually high activity in splitting esculin. In this case the positive test was due to an increase in cellobiase activity.

Enzyme levels in mutants and wild types: Extracts of cell-1

gluc-1 strain exhibited high levels of cellobiase and also cellulase activity and typically low (gluc-1) levels or aryl-beta-glucosidase when grown at either 25 or 31 C.

A cross of the new cell-1, gluc-1 strain was made with a cell-1$^+$, gluc-1$^+$ strain, and ascospores were isolated in order. Extracts from the resulting strains were made and assayed for cellulase and beta-glucosidase activity and the results from one typical ascus are shown in Table I.

TABLE I - Specific enzyme activities in selected cell-1 strains

Strain[a]	Genotype	Cellulase[b]	Cellobiase[c]	Aryl-beta-glucosidase[d]
11-(2-1)	cell-1 , gluc-1$^+$	760	1300	900
11-(2-3)	cell-1 , gluc-1	850	1400	380
11-(2-5)	cell-1$^+$, gluc-1$^+$	40	372	714
11-(2-7)	cell-1$^+$, gluc-1	73	266	267
33-(3-8)	cell-1$^+$, gluc-1	87	233	200
STA	cell-1$^+$, gluc-1	150	285	285

a The 11-(2) strains are ascus isolates from a cross of gluc-1 cell-1 x gluc-1$^+$ cell-1$^+$.
b Cellulase was determined as viscosity change in 1% CMC solution.
c Determined as thermolabile beta-glucosidase at 60 C.
d Determined as thermostable beta-glucosidase at 60 C.

A one gene segration pattern of the hyper-producing cellulase and cellobiase factor cell-1 is indicated. This ascus also shows that cell-1 segregates independently of the gluc-1 character. Strains exhibiting increased cellobiase activity also demonstrated an increased cellulase activity. Random strains from the same cross also showed a corresponding 1:1 segregation pattern of cell-1 to cell-1$^+$ and independence of the gluc-1 locus.

Strains derived from the above ascus were enzymatically induced at 25°C by incubation for 72 hours in minimal medium plus 10^{-3}M cellobiose. The strains which formerly had low levels of cellobiase (cell-1$^+$) now showed high levels compared to those of cell-1 strains. The cell-1 strains increased only slightly in cellobiase activity when induced. Aryl-beta-glucosidase activity increased in activity in all cell-1 strains only when induced by exogenous cellobiose.

To determine the dominance relationship of cell-1 to its cell-1 allele, heterocaryons (Beadle and Coonradt, 1944) were formed in which nuclei containing cell-1 or cell-1^+ genes were present in nearly equal numbers. The cell-1 character was recessive and failed to evoke high levels of cellobiase or cellulase in the presence of cell-1^+ nuclei. These heterocaryon experiments suggest that the cell-1 mutation is regulatory in function. Its action in this respect is similar to the i gene studied in E. coli that acts to produce constitutive levels in at least two enzymes and is also recessive in the presence of i^+ (Jacob and Monod, 1961).

There are now apparently two types of regulatory genes for the beta-glucosidases of Neurospora. The gluc-1 gene causes low aryl-beta-glucosidase production and is dominant in heterocaryons. The cell-1 gene produces constitutively high levels of cellobiase and cellulase but is recessive in heterocaryons with cell-1^+. The linkage relationship of these genes is currently being studied and should contribute to an understanding of their function.

References

Beadle, G.W. and Coonradt, V.L. Genetics 29, 291 (1944).
Eberhart, B.M., Cross, D.F. and Chase, L.R. J. Bacteriol. 87, 761 (1964).
Eberhart, B.M. J. Cell. Comp. Physiol. 58, 11 (1961).
Gornall, A.G., Bardowill, C.J. and David, M.M. J. Biol. Chem. 177, 751 (1949).
Horowitz, N.H. and Shen, H. J. Biol. Chem. 197, 513 (1952).
Jacob, F. and Monod, J. J. Mol. Biol. 3, 318 (1961).
Mahadevan, P.R. and Eberhart, B.M. Arch. Biochem. Biophys 108, 22 (1964).
Reese, E., Siu, R.G.H. and Levinson, H.S. J. Bacteriol. 59, 485 (1950).
Vogel, H.J. Microb. Genet. Bull. 13, 42 (1956).